DEVELOPMENT OF GMO PROBIOTICS-PREBIOTICS CONJUGATED WITH NANO POLYMERS AND THEIR EFFICACY AGAINST GUT-BASED PATOGENS

Project report submitted to Sister Nivedita University in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE
IN
BIOTECNOLOGY

Submitted By
PAYAL SAHA
Registration ID: 230020304421
Enrollment ID: 2322210002030

Advisor
Mr. Mayukh Bose

Director & HOD of Bose Informatics

School of Health Science and Translational Research
Sister Nivedita University
Kolkata

June 2025

BOSE INFORMATICS

c/o B Info Sol Pvt. Ltd.
Ph: 7439673045 / 7439733708
Website: www.boseinformatics.com
Email: informaticsbose@gmail.com
Address: 147/2 Feeder Road, Belgharia, Kolkata-700056

REF: BI/464/024/300

REG: B52200322000447

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OF COMPLETION

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Payal

Saha

Who has completed a 90-days internship in DEVELOPMENT OF GMO PROBIOTICS-PREBIOTICS CONJUGATED WITH NANO POLYMERS AND THEIR EFFICACY AGAINST GUT-BASED PATHOGENS from 12th February 2025 to 20th May 2025 at our premises. We proudly present this certificate for your hard work, dedication, sincerity, perseverance and punctuality. We wish you all the best in your future endeavours.

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I hereby declare that the work which is being presented in this dissertation entitled "**DEVELOPMENT OF GMO PROBIOTICS-PREBIOTICS CONJUGATED WITH NANO POLYMERS AND THEIR EFFICACY AGAINST GUT-BASED PATOGENS**" in partial fulfilment of the requirements for the award of the degree of Masters of Science in Biotechnology is an authentic record of my own work carried out during the period from 2023 to 2025 under the supervision of Dr. Som Subhra Thakur Department of Biotechnology.

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Registration no.: 230020304421

Department: Biotechnology

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DG Block - 1/2, Action Area I, New Town, Kolkata - 700156, West Bengal, India.

DEPARTMENT OF BIOTECHNOLOGY & DEPARTMENT OF MICROBIOLOGY
জৈব প্রযুক্তি বিভাগ ও অণুজীব বিদ্যা বিভাগ

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This is to certify that the project report titled "DEVELOPMENT OF GMO PROBIOTICS-PREBIOTICS CONJUGATED WITH NANO POLYMERS AND THEIR EFFICACY AGAINST GUT-BASED PATHOGENS", submitted by Ms. Payal Saha (Registration No. 230020304421 Enrolment No. 2322210002030) to the Department of Microbiology/Biotechnology, School of Health Science and Translational Research at Sister Nivedita University, Kolkata, is a bona fide record of research conducted by him/her for the partial fulfillment of the requirements for the Master of Science degree.

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Signature of Head of the Department

Seal

Head
Department of Biotechnology
Sister Nivedita University
Block DG-1/2, Action Area 1
New Town, Kolkata-700 156

DEVELOPMENT OF GENETICALLY MODIFIED ORGANISM PROBIOTICS-PREBIOTICS CONJUGATED WITH NANO POLYMERS AND THEIR EFFICACY AGAINST GUT-BASED PATHOGEN

A Project report submitted to Sister Nivedita University in partial fulfilment of the requirement for the degree of

MASTER OF SCIENCE IN BIOTECHNOLOGY

Submitted By

Payal Saha

Registration ID: 230020304421

Enrolment ID: 2322210002030

Advisor

MR. Mayukh Bose

BOSE INFORMATICS

147/2, Feeder Road

Beehive Garden, Belgharia

Kolkata, West Bengal 700056

DECLARATION

I hereby declare that the project entitled “DEVELOPMENT OF GENETICALLY MODIFIED ORGANISM PROBIOTICS-PREBIOTICS CONJUGATED WITH NANO POLYMERS AND THEIR EFFICACY AGAINST GUT-BASED PATHOGEN” is an original piece of work carried out by me under the guidance of Mrs Mayukh Bose, which has been submitted to BOSE INFORMATICS as a 3 months project report.

The project report submitted, is different from any other similar titles to this date. The information has been collected from genuine and authentic sources. References made to the work of others have been cited and listed in the reference section.

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COLLEGE: SISTER NIVEDITA UNIVERSITY

PLACE: NEWTOWN KOLKATA

DATE: 06.06.2025

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ABSTRACT

The escalating threat of antibiotic resistance necessities innovate strategies to combat gut-based pathogens. This research explores the development and efficacy of a novel therapeutic platform that integrates genetically modified organism (GMO) probiotics and prebiotics conjugated with nano-polymers. This Syn-biotic-nanoparticle conjugate represents a next-generation approach to specifically target and eliminate enteric pathogens while promoting a healthy gut microbiome.

This study focuses on the genetic modification of probiotic strains, such as Lactobacillus, to enhance their natural antimicrobial properties. Genes encoding for specific bacteriocins, antimicrobial peptides, and enzymes capable of degrading pathogenic biofilms are introduced and overexpressed. These GMO probiotics are then co-encapsulated with a selective prebiotic, such as inulin, within a biocompatible and biodegradable nano-polymer matrix. The nano-polymer shell serves a dual purpose: it protects the probiotic-prebiotic payload from the harsh acidic environment of the stomach, ensuring targeted release in the intestines, and its surface can be functionalized with ligands that specifically bind to receptors on gut pathogens.

Our studies demonstrate the superior efficacy of this nano-conjugated Syn-biotic system compared to individual components. The targeted delivery mechanism significantly increases the concentration of the GMO probiotic at the site of infection, leading to enhanced pathogen inhibition and displacement. The prebiotic component selectively promotes the growth and activity of the engineered probiotic, further amplifying its therapeutic effect. Our findings indicate a significant reduction in the colonization and virulence of key gut pathogens, including listeria sp and Escherichia coli.

This study focuses on the possibility of using a synergistic combination of genetic engineering, Syn-biotics, and nanotechnology to create an extremely potent and targeted therapy against gastrointestinal infections. The novel GMO probiotic-prebiotic nano-conjugate offers a promising alternative to conventional antibiotics, with the potential to reduce the burden of infectious diseases and circumvent the challenge of antimicrobial resistance.

INTRODUCTION

Probiotics are beneficial microorganisms like bacteria and yeast, which plays crucial role in maintaining gut health. They help to balance the gut microbiome, potentially improving digestion, boosting immunity, and even influencing overall health.

The Food and Agriculture Organisation (FAO) of the United Nations and the World Health Organisation (WHO) define probiotics as “live microorganisms which when administered in adequate amounts confer a health benefit on the host”. While prebiotics are non-digestible fibres that selectively stimulate the growth of beneficial bacteria.

It is now recognised that the human gut microbiota may play a role in the development of metabolic diseases including obesity, diabetes and inflammatory bowel disease.

The combined use of probiotic-prebiotic, synergistic combination (Syn-biotics) faces significant challenges, including poor viability of probiotics strains in the acidic stomach environment, limited colonization ability, and non-specific action against pathogens and thus are genetically modified like Lactobacillus strains modified to produce bacteriocins.

Lactobacillus strains are widely used in probiotics. These probiotic strains could carry acquired antibiotic resistant genes that could be transferred to pathogenic bacteria in the gut or environment. Thus testing for Vancomycin resistance is crucial to ensure the safety of the product. The glycopeptide antibiotic Vancomycin was formally designated as last resort for the control of several resistant Gram-positive bacteria. It was successfully applied to treat severe infections of methicillin resistant S. Aureus. Many strains of lactobacillus are intrinsic strain with vancomycin.

Nano-polymers which are biocompatible and biodegradable polymeric materials on the nanoscale, can be used to encapsulate, protect, and deliver probiotics and prebiotics to the intestines with high precision.

The integration of GMO probiotics, prebiotics, and nano polymers into a unified platform represents a cutting-edge strategy for combating gut-based pathogens. These **bio-nano conjugates** offers therapeutic effects – rebalancing the gut microbiota, directly inhibiting pathogenic microbes, enhancing mucosal immunity, and minimizing systemic side effects.

REVIEW OF LITERATURE

Enhanced probiotics can take over binding sites and consume resources, leaving less room for bacterial growth. Genetically engineer strains can produce elevated levels of bacteriocins, acids, and hydrogen peroxide which suppress invading microbes.

Studies show that these engineered solutions are effective in decolonizing antimicrobial-resistant bacteria in the gut. Syn-biotics with nano- prebiotics have been shown to reduce pathogenic E. coli infections and strengthen the gut lining in mouse models.

A notable strain, E. coli Nissle 1917, has been modified to produce immune-modulating agents like TGF-β, and when paired with smart coatings (like Eudragit), shows exceptional promise against GI infections caused by Salmonella and environmental toxins.

Another variant producing biosynthetic melanin (EcN-Mel) was shown to combat ulcerative colitis by shifting the gut microbiota toward more helpful strains like Lactobacillus and reducing harmful Proteobacteria.

Next-Generation Probiotics (NGPs): This new class of probiotics goes beyond food and supplements. Designed using tools like synthetic biology and gene editing, NGPs are tailored for pharmaceutical-grade applications and hold potential for personalized gut therapies against chronic conditions.

Syn-biotics = Probiotics + Prebiotics: When used together, they create a synergistic effect. Prebiotics not only support the introduced probiotics but also boost the native beneficial bacteria already living in the gut.

Defence Against Drug-Resistant Pathogens: Some studies suggest Syn-biotics can help "crowd out" resistant bacteria by strengthening colonization resistance. Prebiotics also encourage the production of short-chain fatty acids (SCFAs), which acidify the gut and make it a less hospitable place for pathogen.

Nano-polymers help encapsulate sensitive probiotics and prebiotics, protecting them from digestive stressors like stomach acid, bile salts, and enzymes—ensuring more live microbes survive to reach the intestines.

CULTURING OF LACTOBACILLUS

For the preparation of the sample, lactobacillus strain was used. The strain was sourced out as a lab repository.

Cultivation of lactobacillus:

Medium: MRS media was used for the cultivation as it is considered to be ideal for bacterial growth.

Preparation of MRS media:

Table 1: MRS Broth

Ingredients:	For 10ml Solution (µg)
• Protease peptone	0.1
• Beef extract	0.1
• Yeast extract	0.05
• Dextrose	0.2
• Tween 80	0.01
• Ammonium citrate	0.02
• Sodium acetate	0.05
• Magnesium sulphate	0.001
• Manganese sulphate	0.0005
• Dipotassium hydrogen phosphate	0.002

Procedure:

1. Weigh all the ingredients
2. Addition of 10ml distilled water
3. Heating it until the broth is warm and salts have dissolved
4. Wait until luke warm for the bacteria; inoculation

After re-culturing of bacteria in MRS media it was sealed and incubated overnight at 37ºC in a shaker, until the OD reaches 0.6. it was then stored at 4ºC.

BIOCHEMICAL TESTS FOR LACTOBACILLUS

The sample was tested for identification and characterisation

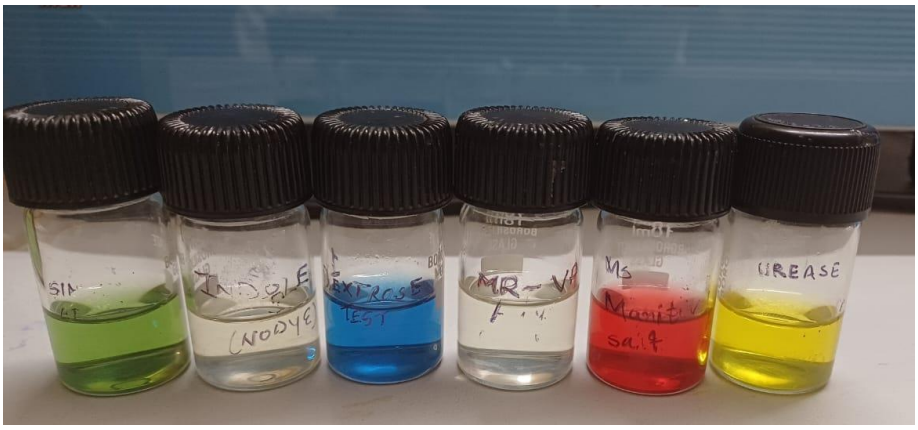

Fig 1: Identification of Lactobacillus sp by Biochemical tests

- MANNITOL SALT AGAR: This is not generally done for lactobacillus species because it is a selective medium that favours the growth of halophilic bacteria. Bacteria tested positive for this would show a change in colour from red to yellow.

  INGREDIENTS:

  1. Protease peptone
  2. Beef extract
  3. Sodium chloride
  4. D-Mannitol
  5. Phenol red (dye)

  Result: negative result

Fig 2: Colour change shows negative results of Mannitol test

- SIMMONS CITRATE MEDIA: This test is used to determine if the bacteria can utilise citrate as its sole source of carbon. Lactobacillus species are generally considered citrate- negative. If there is a colour change from green to blue it is positive (producing alkaline biproducts) if not then colour does not change.

  INGREDIENTS:

  1. Magnesium sulphate
  2. Ammonium dihydrogen phosphate
  3. Dipotassium phosphate
  4. Sodium citrate
  5. Sodium chloride
  6. Bromothymol blue (dye)

  Result: negative, no change in colour.

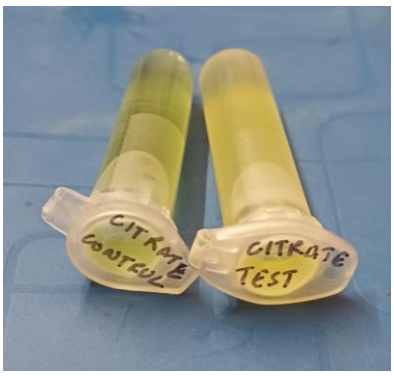

Fig 3: No colour change shows negative Simmon Citrate

INDOLE TEST: this test determines if the bacteria can metabolize the amino acid tryptophan and produce indole. Lactobacillus typically doesn't produce indole. While some lactobacillus strains are known to be indole positive. The positive indole test is indicated by the development of cherry red colour in the top layer after adding Kovac's reagent.

INGREDIENTS:

1. Tryptone
2. NaCl
3. Kovac's reagent

Result: shows a positive test with red colour ring at top.

Fig 4:Red ring shows positive Indole test

UREASE TEST: This test relies on the ability of bacteria to utilize urea, a nitrogen containing compound, into ammonia and carbon dioxide. Lactobacillus strains are urease negative.

INGREDIENTS:

1. Potassium dihydrogen phosphate
2. Dipotassium hydrogen phosphate
3. Yeast extract
4. Phenol red (dye)

Result: negative, colour remains yellow.

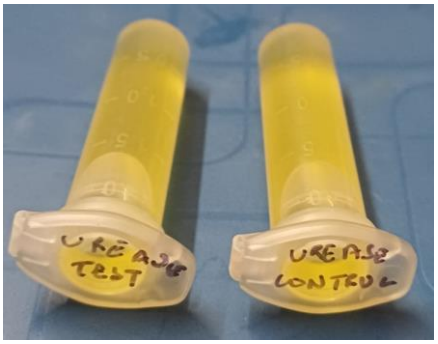

Fig 5: No colour change shows negative Urease test

MR-VP TEST: The production of lactic acid is detected by MR (lactobacillus fermentation) while the production of acetoin is detected by VP. Lactobacillus is generally positive for MR but negative for VP

INGREDIENTS:

1. Dextrose/ Glucose
2. Dipotassium phosphate
3. Peptone

Result: positive for MR and negative for VP.

Fig 6: MR-VP Test

DEXTROSE/ GLUCOSE TEST: It is done to identify the ability of lactobacillus to ferment glucose. A positive result, indicated by acid production confirms glucose fermentation.

INGREDIENTS:

1. Tryptone
2. Sodium chloride
3. Dipotassium hydrogen phosphate
4. Glucose/ dextrose
5. Bromothymol blue (dye)

Result: positive, yellow colour.

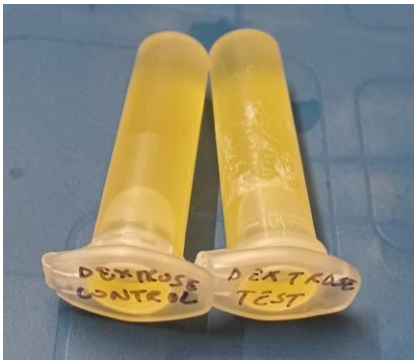

Fig 7: Yellow colour shows Positive Dextrose/ Glucose Test

PREPARATION OF SAMPLE WITH PREBIOTIC

Store bought inulin was used as prebiotic in this experiment. 1% inulin was added to the precultured bacteria in the MRS media and Optical Density was checked with an hour interval. The growth was monitored with every hour interval for several hours, then kept overnight for the last reading to check the growth.

The standard was lactobacillus in MRS broth without the inulin.

Table 2: LACTOBACILLUS OD IN MRS MEDIA:

TIME	WITH INULIN	WITHOUT INULIN
1ST HOUR	0.14	0.03
2ND HOUR	0.21	0.1
3RD HOUR	0.30	0.25
4TH HOUR	0.42	0.31
OVERNIGHT	0.97	0.88

ANTI-MICROBIAL RESISTANCE OF LACTOBACILLUS

Vancomycin is a glycopeptide antibiotic used to treat severe infection by gram positive bacteria. The glycopeptide antibiotic that inhibits the polymerization of peptidoglycans in the bacterial cell wall is known as Vancomycin. Vancomycin is only active against gram-positive bacteria.

In this experiment, we used 1% vancomycin for the resistance of the bacteria(5mg/5ml). For understanding the minimum concentration of vancomycin needed for resistance we give dosages and check the OD. Dosage started from 200µl, 400µl, 600µl, 800µl, 1ml.

DOSE 1:

C1= 1mg/ml

C2= 100µl

V2= 10ml

C1V1= C2V2

$$V1= \frac{c2\ x\ v2}{c1}$$

V1= (100µg x 10ml)/5000µg =0.2ml (200µl)

10ml MRS +1ml lactobacillus with inulin + 200µl vancomycin.

Then incubated overnight to check result.

The OD the next morning 0.84.

DOSE 2:

C1= 5mg/5ml (5000µg)

C2= 200µl

V2= 10ml

C1V1= C2V2

$$V1 = \frac{c2\ x\ v2}{c1}$$

V1= (200µg x 10ml)/5000µg =0.4ml (400µl)

10ml MRS +1ml lactobacillus with inulin + 400µl vancomycin.

Then checked for bacterial growth in each hour.

Table 3: Observation of OD after Dose 2

TIME	OD
1ST HOUR	0.37
2nd HOUR	0.57
3RD HOUR	0.7

Stored overnight at 4°c

DOSE 3:

C1= 5mg/5ml (5000µg)C2= 300µl

V2= 10ml

C1V1= C2V2

$$V1 = \frac{c2\ x\ v2}{c1}$$

V1= (300µg x 10ml)/5000µg =0.4ml (600µl)

10ml MRS +1ml lactobacillus with inulin + 600µl vancomycin.

Then checked for bacterial growth in each hour.

Table 4: Observation of OD after Dose 3

TIME	OD
1ST HOUR	0.18

2nd HOUR	0.44
3RD HOUR	0.64

Stored overnight at 4°c

DOSE 4:

C1= 5mg/5ml (5000µg)

C2= 400µl

V2= 10ml

C1V1= C2V2

$$V1= \frac{c2\ x\ v2}{c1}$$

V1= (400µg x 10ml)/5000µg =0.4ml (800µl)

10ml MRS +1ml lactobacillus with inulin + 800µl vancomycin.

Then checked for bacterial growth in each hour.

Table 5: Observation of OD after Dose 4

TIME	OD
1ST HOUR	0.1
2nd HOUR	0.31
3RD HOUR	0.52

Stored overnight at 4°c

DOSE 5:

C1= 5mg/5ml (5000µg)

C2= 500µl

V2= 10ml

C1V1= C2V2

$$V1 = \frac{c2\ x\ v2}{c1}$$

V1= (500µg x 10ml)/5000µg =0.5 (1000µl)

10ml MRS +1ml lactobacillus with inulin + 1000µl vancomycin

Then checked for bacterial growth in each hour.

Table 6: Observation of OD after Dose 5

TIME	OD
1ST HOUR	0.08
2nd HOUR	0.21
3RD HOUR	0.46

Stored overnight at 4°c

With each increase in dosage concentration the growth of bacteria is effected thus showing that the bacteria is resistant to vancomycin.

EXTRACTION 0F METABOLTIES

After making the bacteria resistant to antibiotic the extraction of metabolites is done for nano-coating it with chitosan.

The metabolites contain bacteriocin and all the intracellular components.

Procedure:

1. MRS media is freshly prepared
2. The vancomycin resistant lactobacillus is inoculated in MRS media
3. Incubate at 37° C in a shaker.
4. Check the OD after few hours
5. After OD reached 2.0 it is ready for metabolite extraction
6. Each Eppendorf is filled with sample and placed in the centrifuge
7. Run centrifuge at 10,000 for 3 min
8. Collect the supernatant containing the cell metabolites.
9. Resuspend the pellets in a different broth for future use

The CFS metabolites contain the bacteriocin, lactic acid, antimicrobial peptides, and other molecules contributing to anti-microbial activity.

This is now used for the nanocoating with chitosan.

NANOPARTICLE FORMATION

Chitosan is a linear polysaccharide. It is mainly obtained by alkaline deacetylation of chitin. Chitin is found mainly in the shells of crustaceans and in the fungal cell walls as well as arthropods. Because of the unique structure of CS (the amine group is randomly acetylated and rich in hydroxyl groups), it has shown many properties including antioxidant, anti-bacterial, anti-cancer, non-toxic, edible, and biodegradable effects. CS has been widely used in biology, medicine, cosmetic, food industry, sewage treatment, chromatography, and enzyme immobilization. However, CS can only be soluble in acidic solution (pH $\leq$ 4.6) and it is greatly limited in terms of functionality.

In this experiment we nano-coated lactobacillus bacteria with a chitosan -TPP solution. This enhances their stability and viability, especially in challenging gastrointestinal environments.

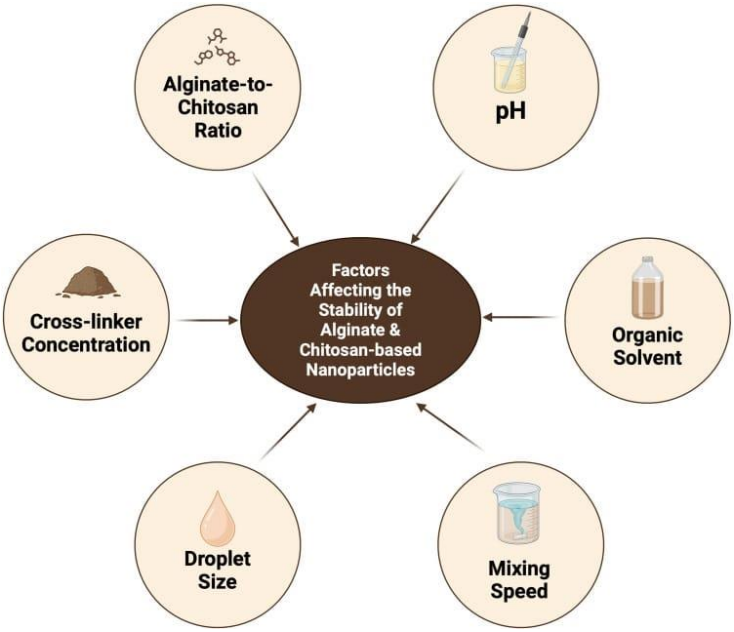

Fig 8: Factors affecting the stability and viability in challenging gastrointestinal environments

I. pH: Alginate and chitosan are sensitive to pH changes. Alginate ionizes in basic conditions, while chitosan ionizes in acidic conditions. This ionization impacts the strength of polyelectrolyte interactions, which are crucial for nanoparticle formation.

II. Crosslinking Concentration: The concentration of crosslinking agents, like calcium ions (for alginate), is required for creating a stable nanoparticle network.

III. Mixing Speed: Increased stirring speed during nanoparticle formation can lead to particle breakup and size reduction.

IV. Droplet Size: In methods like layer-by-layer self-assembly, the size of the droplets influences the thickness of the nanoparticle coating.

V. Organic Solvents: The use of organic solvents, often in co-solvency processes, can impact the overall stability of the nanoparticles.

VI. Alginate and Chitosan Concentration Ratio: The ratio of alginate to chitosan can be adjusted to optimize nanoparticle formation and properties.

VII. Temperature: Temperature can affect the stability of the nanoparticle structure and the release of encapsulated drugs.

VIII. Anions: The presence of anions in the environment can directly impact the size and stability of the nanoparticles.

As chitosan is positively charged, TPP is added as it is a negatively charged anionic polymer that interacts with the positively charged chitosan, forming a cross-linked network that encapsulates the lactobacillus bacteria.

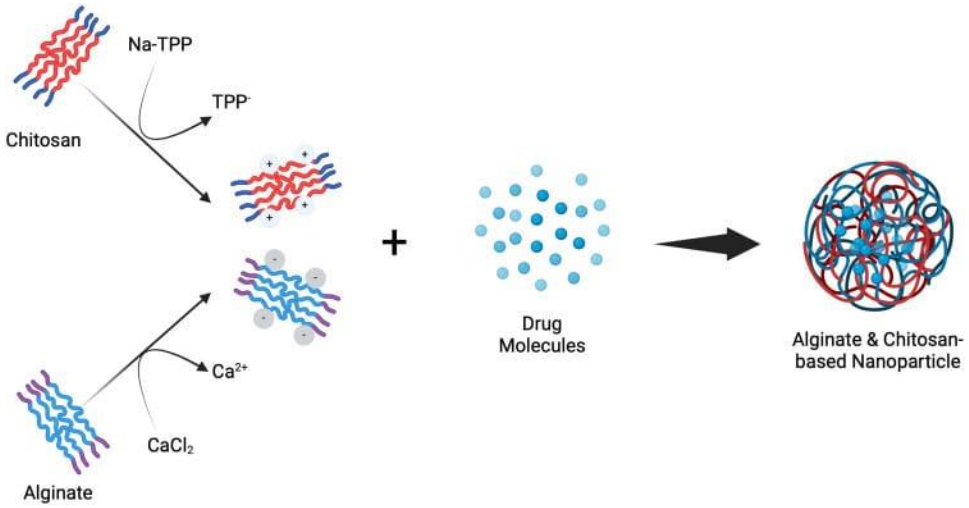

Fig 9: Cross linking of Chitosan with alignate

A. Preparation of Chitosan Solution:

1. 0.1-0.2g of chitosan is weighed.
2. Dissolved in 100ml of 1% (v/v) acetic acid under the magnetic stirrer.
3. It is stired for 6–8 hours until the solution is clear.
4. using 0.1 M NaOH, pH was adjusted to 5.5–6.0 if needed.

B. Preparation of Sodium Alginate Solution:

1. 0.1–0.2 g of sodium alginate is weighed
2. Then dissolved in 100 mL of deionized water.
3. Stirring until completely dissolved.
4. Dissolving the required amount of antibacterial drug in the alginate solution. Stirring until the drug is uniformly dispersed.

C. Preparation of Crosslinking Agent ($CaCl_2$)

1. 0.1–0.2% (w/v) calcium chloride solution is prepared in deionized water.
2. Then Antibacterial Agent was incorporated.

D. Nanoparticle Formation by Ionic Gelation

1. The drug-loaded alginate solution is loaded dropwise into the chitosan solution using a syringe or peristaltic pump under continuous stirring.
2. Continue stirring for 1–2 hours at room temperature.
3. Addition of $CaCl_2$ solution dropwise to promote cross-linking and nanoparticle formation.

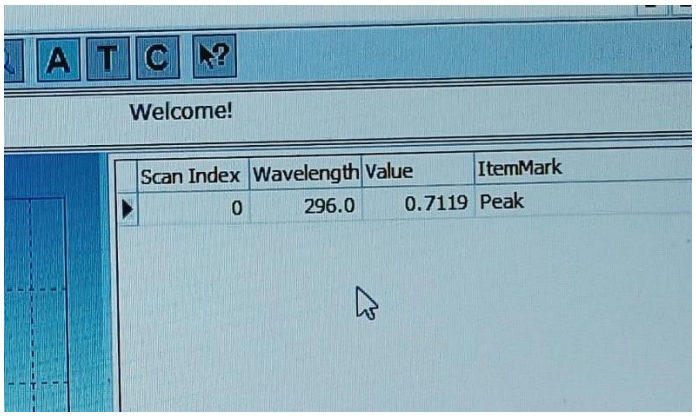

Fig 10: Peak Scene at 296mm Wavelength of Nano-coated Lactobacillus

A distinctive peak in the UV-Vis spectrum indicates the formation of nanoparticle. Thus stating that the drug is now coated with nanobacteria and is a chitosan TPP complex.

It was then stored at 4°c.

CHECKING THE EFFICACY OF THE NANO-COATED LACTOBACILLUS.

While the nano-coated bacteria are stored at 4°c. we will have to check it's efficacy. General gut bacteria includes *Escheria coli, Listeria sp.*

Two Lysogeny Broth Agar plates were made where e.coli and listeria are inoculated for culturing. In 7ml agar plate 1µl lab repository e.coli and listeria are added to their respective plates, sealed and incubated until growth could be seen.

After visible growth of both the bacteria, they are re-cultured in LB broth again and incubated until it reached 0.6 OD which is essential for MIC.

This definition of MIC (Minimum Inhibitory Concentration) is made up of three important elements: it refers to a threshold for a *minimal* change above which patients perceive themselves as changed (improved or deteriorated).

The MIC number is the lowest concentration (in µg/mL) of an antibiotic that inhibits the growth of a given strain of bacteria.

The MIC is determined by the inoculation of drug in bacterial culture broth and checking the OD periodically to determine the growth. The lower the optical density the more powerful is the drug. It is measure in the UV -Vis spectrophotometer.

When there is a stationary phase on the graph then it is called to be the MIC of the drug i.e the minimum inhibitory concentration

Procedure:

1. Prepare two cultured broth of *Escherichia coli* and *Listeria sp.*
2. They are the standard for the MIC.
3. Addition of nanocoated drug is done with gradually increasing dosage
4. Checking of OD on the UV-Vis spectrophotometer.

MIC OF *ESCHERICHIA.COLI:*

Standard OD= 0.56

Table 7: Observing Drug OD against standard for MIC

STANDARD	DOSAGE	TIME(H)
0.68	0.64	1
0.72	0.68	2
0.8	0.75	3

MIC OF *LISTERIA SP:*

Standard OD= 0.58

Table 8: Observing Drug OD against standard for MIC

STANDARD	DOSAGE	TIME(H)
0.66	0.45	1
0.79	0.57	2
0.86	0.63	3

IC 50

IC50, which stands for half-maximal inhibitory concentration, is a measure of the potency of a substance in inhibiting a specific biological function, including bacterial growth. It's the concentration of that substance required to reduce the biological process or response by half. In the context of bacteria, IC50 can be used to assess the effectiveness of antibiotics or other antimicrobial agents.

IC 50 OF *ESCHERICHIA.COLI:*

Standard OD= 0.57

Table 9: Observing Drug OD against standard for IC50

STANDARD	DOSAGE	TIME(H)
0.66	0.34	1
0.71	0.4	2
0.72	0.35	3

IC 50 OF *LISTERIA SP:*

Standard OD= 0.61

Table 10: Observing Drug OD against standard for IC 50

STANDARD	DOSAGE	TIME(H)
0.67	0.32	1
0.7	0.58	2
0.73	0.39	3

ZONE OF INHIBITION:

A zone of inhibition refers to the area surrounding an antimicrobial agent on a culture plate where bacterial growth is prevented. The antibiotic has effectively inhibited or killed the bacteria which is shown by a clear surace. This zone is a key indicator of the antibiotic's effectiveness against a specific bacterial strain.

Procedure:

1. LB agar plate is prepared.
2. Then waited until it was solidified
3. Until surface is dry, bacteria is spread well on the agar surface
4. A Well is dug on the solidified agar plate with a cork borer
5. Fill the well with the drug.
6. Incubate overnight for growth
7. Check for zone of inhibition the next day or after 18 hours

In this experiment we saw the effect of nanocoated drug on two bacteria:

a. Escherichia coli:

- The agar plate was spread with e.coli (from lab repository)
- Then well is dug
- The drug is given in the well until the well is full till brim
- Plates were sealed and then incubated
- Zone of inhibation was checked.

Fig 11: A clear zone could be seen on the plate.

CALCULATION:

Outer circle= 1.7+1.7+1.5 =4.9

Average= 4.9/3 =1.63

Inner circle= 0.9+0.8+0.8 =2.5

Average= 2.5/3 = 0.83

Thus, zone of inhibition = 1.63- 0.83 =0.8cm

b. Listeria sp:

- The agar plate was spread with Listeria (from lab repository)
- Then well is dug
- The drug is given in the well until the well is full till brim
- Plates were sealed and then incubated
- Zone of inhibation was checked.

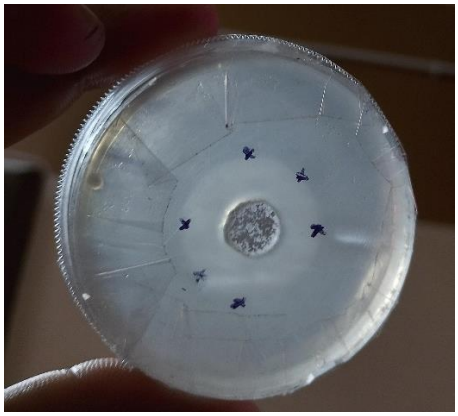

Fig 12: clear zone of inhibition could be seen.

CALCULATION:

Outer circle= 2.2+ 2+2.3= 6.5

Average= 6.5/3 = 2.1

Inner circle= 0.8+0.8+0.8 =2.4

Average= 2.4/3 = 0.8

Thus, zone of inhibition = 2.1- 0.8 = 1.3 cm

RESULT

Lactobacillus is a part of gut bacteria which helps in preservation of the gut microbiome along with other bacteria. Thus culturing and antimicrobial resisting lactobacillus proved us to be beneficial. Gut microbiome works better when conjugates with probiotics. Probiotics are living microorganisms which stays in gut for better health.

Genetically modified probiotics is engineered to produce compounds important for recent studies. It forms antimicrobial peptides, bacteriocins, or expresses proteins that interact with pathogens, preventing their colonization. Lactoferrin and lysozyme are formed when lactobacillus is genetically modified.

Lactoferrin is an iron binding glycoprotein, playing crucial role in the body's defence mechanism. It acts as natural protective agent with antiviral, anti-inflammatory, and antiviral properties.

Nanocoated Lactobacillus probiotic-prebiotic conjugate protects bacteria from harsh environmental conditions of the gastro intestinal tract. *Lactobacillus Rhamnosus* is effective for preventing and treating antibiotic associated Diarrhea particularly in children. *Lactobacillus acidophilus* is a common probiotic balances gut flora and helps with digestive issues for immune functions.

After culturing of lactobacillus in MRS media, the culture was plated to see grown on the solid media showing the presence of bacteria in broth. Addition of inulin to lactobacillus worked as food and increased efficacy of the lactobacillus. As we saw earlier, Strain conjugated with prebiotic, i.e. Inulin was growing more rapidly than the standard broth.

Lactobacillus was made antibiotic resistant with Vancomycin and we saw considerable decrease in the growth curve of the bacteria.

While coating lactobacillus with Chitosan and TPP, we faced two challenges:

1. Adjusting the PH: Chitosan needs Acidic environment to dissolve which was provided by acetic acid. While the conjugate will form at a basic environment thus maintaining the PH at 5.5 to 6 is very crucial which can be done by the addition of Sodium Hydroxide.
2. Managing the charge: Chitosan is positively charged. TPP is negatively charged. For conjugate to form this charge must be managed otherwise we will not get clear nanocoated sample. $CaCl_2$ was added as a stabilizer. It helped dispersion of nanoparticles uniformly, prevent aggregation and created a stable protected layer. Also provided mechanical strength and flexibility of the coating.

DISCUSSION

As lactobacillus is found in the gut it is a gut pathogen. And probiotics play a very important role in maintaining the gut health humans. Recent studies have shown us how wider range of health benefits from probiotics, including immune functions, mental health, and even cancer prevention. There are still many ongoing projects focusing on the finding new probiotic strains and delivery methods, and their importance in gut microbiome. Prebiotic when infused probiotic is called symbiotic. Researchers are still looking for link between gut microorganisms and health conditions such as, allergies, irritable bowel syndrome, depression, etc. Recent studies have also shown that prebiotic and probiotics have no or rare side effects but still is not safe for everyone. Nanocoating of lactobacillus is done for the enhancement of drug delivery and functionality. It helps in survival of the hard gastrointestinal environment by improving their ability to adhere to internal mucosa. Nanocoating of lactobacillus with chitosan and TPP is quiet challenging as chitosan takes time to dissolve in the media and should have an acidic ph. TPP is negatively charged which allows the formation of the nano-complex. Sodium alginate is used to further control the charge on the media. Calcium chloride is added along with it. The ca+ ion disperses in the media giving it positive charge for nano formation. Another important aspect to notice while doing nano is maintenance if ph. Chitosan dos not dissolve in basic media but acidic. Thus gradual addition of acetic Acid is done to dissolve. Then the Ph needs to be set at 5.5 to 6 for the rest of the time.

CONCLUSION

The development of genetically modified (GM) probiotics and prebiotics combined with nano-polymers is emerging as an exciting and innovative approach in the fight against gut-related infections. By leveraging the combined strengths of biotechnology and nanotechnology, this strategy enhances the effectiveness of probiotics in ways we couldn't achieve before. Through genetic modification, probiotics can be customized to better perform their beneficial functions. This includes producing higher levels of antimicrobial compounds, sticking more effectively to the gut lining (which helps them outcompete harmful microbes), and delivering specific therapeutic molecules—like enzymes or cytokines—right where they are needed in the gut.

When probiotics are paired with prebiotics (known as Syn-biotics), the combination works better than either component alone. As prebiotics are non-digestible compounds that help "feed" the good bacteria in your gut, including the ones you take as supplements.

Many engineered probiotics can be designed to produce higher amounts of bacteriocins and organic acids, which directly inhibit or kill harmful microbes. These advanced probiotics helps in re-establish a healthy balance of gut bacteria (known as eubiosis), especially after it’s been disrupted by illness, antibiotics, or poor diet.

Because of their targeted delivery and local action, they may reduce the side effects often seen with traditional treatments like antibiotics.

Current Challenges and What Comes Next

Safety First: Genetic modification naturally raises concerns. Scientists need to ensure these engineered probiotics are stable, won’t transfer genes to other microbes, and won’t unintentionally harm the gut ecosystem.

Regulatory Complexity: The rules around GMOs and nanotechnology in medicine are still developing. We need clear, standardized guidelines for testing and approval.

Manufacturing & Costs: These products are complex and expensive to develop. Scaling them for commercial use in a cost-effective way is still a work in progress.

Clinical Trials: Most of the evidence we have is from lab studies or animal models. We need high-quality human trials to prove these therapies are both effective and safe for widespread use.

Better Understanding Needed: We still don't fully understand all the ways these engineered microbes interact with our existing gut bacteria, our gut lining, and our immune system. More research is needed to map out these relationships.

Bringing together genetic engineering, probiotic science, and nanotechnology offers a cutting-edge way to treat gut-related illnesses more precisely and effectively. These next-generation Syn-biotics could represent a major shift in how we support gut health—delivering good bacteria more efficiently, targeting harmful microbes more directly, and possibly reducing the need for traditional antibiotics.

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